Test Run Reports

Shipboard Tests of the Bawat Ballast Water Treatment System for Type Approval according to Regulation D-2 and the relevant IMO Guideline (G8)

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Test Run Report

1st and 2nd Shipboard Tests of the Bawat Ballast Water Treatment System for Type Approval according to Regulation D-2 and the relevant IMO Guideline (G8)

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01-10-2013

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1. Introduction

This is the summary report of the first two test runs to test the performance of the Bawat ballast water treatment system conducted in Port Khalid near Dubai (United Arab Emirates) in September 2013.

2. Biological sample processing

The samples were processed as described in the Bawat QAPP, Version 5.2, August 2013 (Gollasch & Lüthke Christensen 2013).

3. Results

3.1. Environmental parameters

The sample processing revealed environmental parameters as expected to occur in the ballast water uptake region, although it was noted that the water temperature and salinity were higher than average for the season.

3.2. Biological results

The data presented below in the test cycle reports are mean values of all measurements. For individual biological counts see the Appendices.

The number of organisms in the uptake water met the challenge water conditions for tests of treatment systems as outlined in the IMO Test Guideline G8 (RESOLUTION MEPC.174(58) Adopted at IMO on 10 October 2008 GUIDELINES FOR APPROVAL OF BALLAST WATER MANAGEMENT SYSTEMS).

The PAM measurements conducted in the hotel room in Dubai and at NIOZ show comparable results (see Appendix 7). This is especially the case for the samples with high cell content (uptake, discharge of the control test water).

3.2.1. Test run 1

The test was done with a ballast water uptake on 05.09.2013, 10:06 – 12:12, in Khalid Port, near Dubai. Next day the ballast water was sampled at discharge: 06.09.2013, 09:58 - 13:13.

The holding time of ballast water between uptake and discharge ca. 24 hours.

Water quality and number of organisms in uptake and discharge water (nd = not determined, P = phytoplankton, Z = zooplankton)

Parameter	**Unit**	**Uptake water*** **					**Discharge water**															
		before treatment			**aver.**	**IMO DNV**	**control**			**aver.**	**IMO DNV**	**treated**									**aver.**	**IMO DNV**
		#1	**#2**	**#3**	**#1-3**		**#1**	**#2**	**#3**	**#1-3**		**#1**	**#2**	**#3**	**#4**	**#5**	**#6**	**#7**	**#8**	**#9**	**#1-9**	
Temperature	°C	38.0	38.0	38.0	38.0	-	38.5	38.5	38.5	38.5	-	53.1	53.1	53.1	53.1	53.1	53.1	53.1	53.1	53.1	53.1	-
Salinity	PSU	42.1	42.1	42.1	42.1	-	42.2	42.2	42.2	42.2	-	41.6	41.6	41.6	41.6	41.6	41.6	41.6	41.6	41.6	41.6	-
POC **	mg/l	12.8			-	-	12.8			-	-	5.8									-	-
TSS **	mg/l	22.4			-	-	23.2			-	-	12.6									-	-
Sample vol. >50 µm	Litres	1321	1303	1520	1381	>1000	1200	1160	1264	1208	>1000	1594	1603	-	1625	1602	-	1654	1748	-	1638	>1500
Sample vol. 50-10 µm	Litres	5-6	5-6	5-6	5-6	>5	5-6	5-6	5-6	5-6	>5	5-6	5-6	5-6	5-6	5-6	5-6	5-6	5-6	5-6	5-6	>5
Sample vol. bacteria	Litres	1	1	1	1	>0,5	1	1	1	1	>0.5	1	1	1	1	1	1	1	1	1	1	>0,5
Organisms >50µm	org./1m³	4807	3599	2191	3532	>90	917	776	665	786	>10	nd	nd	-	nd	nd	-	nd	nd	-	nd	<10
	time [h]	<4h	<4h	<4h		<6h	<6h	<6h	<6h			<4h	<4h	-	<4h	<4h	-	<5h	<5h	-		<6h
Organisms 10-50µm*	P org./1ml	179	137	93	137	>90	56	93	87	79	>10	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	<10
	Z org./1ml	1.2	2.2	1.8	1.7		0.5	0.3	0.6	0.5		nd	nd	-	nd	nd	-	nd	nd	-	nd	
	time [h]	PAM <6h, NIOZ 12.09.13				<6h	PAM <8h, NIOZ 12.09.13					PAM <8h, NIOZ 12.09.13										<6h
Escherichia coli	cfu/100ml	19	14	30	21	-	3	nd	8	3.7	-	4	nd	nd	nd	2	nd	nd	nd	nd	0.7	<250
	time [h]	<4h	<4h	<4h	<4h	<6h	<4h	<4h	<4h	<4h	<6h	<4h	<4h	<4h	<4h	<4h	<4h	<4h	<4h	<4h	<4h	<6h
Intestinal *Enterococci*	cfu/100ml	1	nd	150	50.3	-	120	50	30	66.7	-	20	20	9	10	6	4	2	5	nd	8.4	<100
	time [h]	<4h	<4h	<4h	<4h	<6h	<4h	<4h	<4h	<4h	<6h	<4h	<4h	<4h	<4h	<4h	<4h	<4h	<4h	<4h	<4h	<6h
*Vibrio cholerae****	cfu/100ml	nd	nd	nd	nd	-	nd	nd	nd	nd	-	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	<1
	time [h]	IBEN 13-23.09.13				<6h	IBEN 13-23.09.13					IBEN 13-23.09.13										<6h

* Analyzed at NIOZ, Texel, The Netherlands, except zooplankton which was processed in the hotel room in Dubai. ** Analysed at IBEN, Bremerhaven, Germany. *** It was agreed with DNV that the water to be treated was sampled during uptake and not the control tank water. All PAM samples were prepared within 6 hours after sampling ended (dark adaptation), uptake samples were measured <6h, discharge samples <8 h.

3.2.2. Test run 2

The test was done with a ballast water uptake on 07.09.2013, 08:23 – 10:26, in Khalid Port, near Dubai. Next day the ballast water was sampled at discharge: 08.09.2013, 06:53 - 10:32.

The holding time of ballast water between uptake and discharge ca. 22 hours.

Water quality and number of organisms in uptake and discharge water (nd = not determined, P = phytoplankton, Z = zooplankton)

Parameter	**Unit**	**Uptake water*** **					**Discharge water**															
		before treatment			**aver.**	**IMO DNV**	**control**			**aver.**	**IMO DNV**	**treated**									**aver.**	**IMO DNV**
		#1	**#2**	**#3**	**#1-3**		**#1**	**#2**	**#3**	**#1-3**		**#1**	**#2**	**#3**	**#4**	**#5**	**#6**	**#7**	**#8**	**#9**	**#1-9**	
Temperature	°C	37.9	37.9	37.9	37.9	-	38.1	38.1	38.1	38.1	-	52.1	52.1	52.1	52.1	52.1	52.1	52.1	52.1	52.1	52.1	-
Salinity	PSU	42.5	42.5	42.5	42.5	-	42.3	42.3	42.3	42.3	-	41.9	41.9	41.9	41.9	41.9	41.9	41.9	41.9	41.9	41.9	-
POC **	mg/l	13.4			-	-	11.2			-	-	12.0									-	-
TSS **	mg/l	22.4			-	-	21.2			-	-	24.4									-	-
Sample vol. >50 µm	Litres	1307	1232	1422	1320	>1000	1200	1128	1469	1266	>1000	1636	1638	-	1793	1823	-	1820	1766	-	1746	>1500
Sample vol. 50-10 µm	Litres	5-6	5-6	5-6	5-6	>5	5-6	5-6	5-6	5-6	>5	5-6	5-6	5-6	5-6	5-6	5-6	5-6	5-6	5-6	5-6	>5
Sample vol. bacteria	Litres	1	1	1	1	>0,5	1	1	1	1	>0.5	1	1	1	1	1	1	1	1	1	1	>0,5
Organisms >50µm	org./1m³	2915	2281	1470	2222	>90	317	284	402	334	>10	nd	nd	-	nd	nd	-	nd	nd	-	nd	<10
	time [h]	<4h	<4h	<4h	<4h	<6h	<6h	<6h	<6h		<6h	<4h	<4h	-	<4h	<4h	-	<4h	<5h	-		<6h
Organisms 10-50µm*	P org./1ml	126	117	82	109	>90	37	51	118	69	>10	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	<10
	Z org./1ml	0.6	0.6	0.7	0.6		0.2	0.3	0.4	0.3		nd	nd	-	nd	nd	-	nd	nd	-	nd	
	time [h]	PAM <6h, NIOZ 13.09.13				<6h	PAM <8h, NIOZ 13.09.13				<6h	PAM <8h, NIOZ 13.09.13										<6h
Escherichia coli	cfu/100ml	260	280	130	223	-	nd	nd	nd	nd	-	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	<250
	time [h]	<4h	<4h	<4h	<4h	<6h	<4h	<4h	<4h		<6h	<4h	<4h	<4h	<4h	<4h	<4h	<4h	<4h	<4h		<6h
Intestinal *Enterococci*	cfu/100ml	20	50	130	66.7	-	30	20	nd	16.7	-	5	nd	nd	nd	2	nd	10	3	3	2.6	<100
	time [h]	<4h	<4h	<4h	<4h	<6h	<4h	<4h	<4h		<6h	<4h	<4h	<4h	<4h	<4h	<4h	<4h	<4h	<4h		<6h
*Vibrio cholerae***	cfu/100ml	nd	nd	nd	nd	-	nd	nd	nd	nd	-	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	<1
	time [h]	IBEN 13-23.09.13				-	IBEN 13-23.09.13				-	IBEN 13-23.09.13										-

* Analyzed at NIOZ, Texel, The Netherlands, except zooplankton which was processed in the hotel room in Dubai. ** Analysed at IBEN, Bremerhaven, Germany. *** It was agreed with DNV that the water to be treated was sampled during uptake and not the control tank water. All PAM samples were prepared within 6 hours after sampling ended (dark adaptation), uptake samples were measured <6h, discharge samples <8 h.

4. Remarks

For both test runs:

- All technical requirements, such as minimum water volumes of the water to be sampled etc. for a valid test according to IMO Guideline G8 or as agreed with DNV prior the tests were met.
- For the organisms above 50 µm and organisms below 50 and above 10 µm in minimum dimension the mean values of the minimum organism intake concentrations and, at discharge of the treated water, D-2 was met.
- For the indicator microbes *Escherichia coli*, Enterococci and *Vibrio cholerae* D-2 was met at discharge.
- All samples were prepared for processing in the hotel room in Dubai and according to the QAPP and G8 paragraph 2.3.34: *The samples should be analysed as soon as possible after sampling, and analysed live within 6 hours or treated in such a way so as to ensure that proper analysis can be performed*.
- For the PAM measurements the phytoplankton samples were prepared for darkness adaptation within 6 hours after sampling and the PAM measurements were completed in maximum 8 hours after sampling.
- It was noted that the natural water temperature during the uptake sampling events was very high (ca. 38 °C), at discharge the temperature of the treated water was above 52 °C. Consequently, the phytoplankton species were not stored in the fridge because and exposure to 6-8 °C fridge temperature would have resulted in a cooling treatment which likely would have caused algae mortality. Room temperature (air conditioned) was already more than 10 °C cooler compared to ambient water conditions so that this was considered an appropriate storage temperature.

Test run 1

- All blank bacteria media experiments conducted were negative.

Test run 2

- The blank *E. coli* media experiment during the uptake test was negative. For the discharge analysis the blank test resulted in 17 *E. coli* cfu in 100 ml (see end of Appendix 4). However, on discharge neither control nor treated water tests showed any colony forming *E. coli* (see tables above). This indicates a possible contamination of the distilled water used for the sample processing. Please note that four unopened bottles of distilled water were used, so that for each uptake and discharge sample processing event a new bottle was used.
- The Enterococci blank test was also positive with 14 cfu in 100 ml and these bacteria were also found in low numbers in the discharge of the control and treated water (see tables above and end of Appendix 6).

5. Temperature logger measurements

To document the sample storage, transport and bacteria incubation conditions temperature loggers were used. The loggers were set to take a temperature measurement every 10 minutes and the following figures show the results. Arrows are used to indicate approximate times for the events as stated in the figure capture. It should be noted that some temperature drops occurred due to opening the incubators for bacteria inspections. This was only done at the end of the incubation time so that the short temperature drop has no effect on the incubation. This becomes very clear in the incubation with the higher temperature (Fig. 2).

The short-term temperature increases in Fig. 4 (phytoplankton storage at room temperature) were due to the storage of additional new water samples of high ambient temperature (Arrows A and C) and treated water on discharge with an even higher water temperature (Arrows B and D).

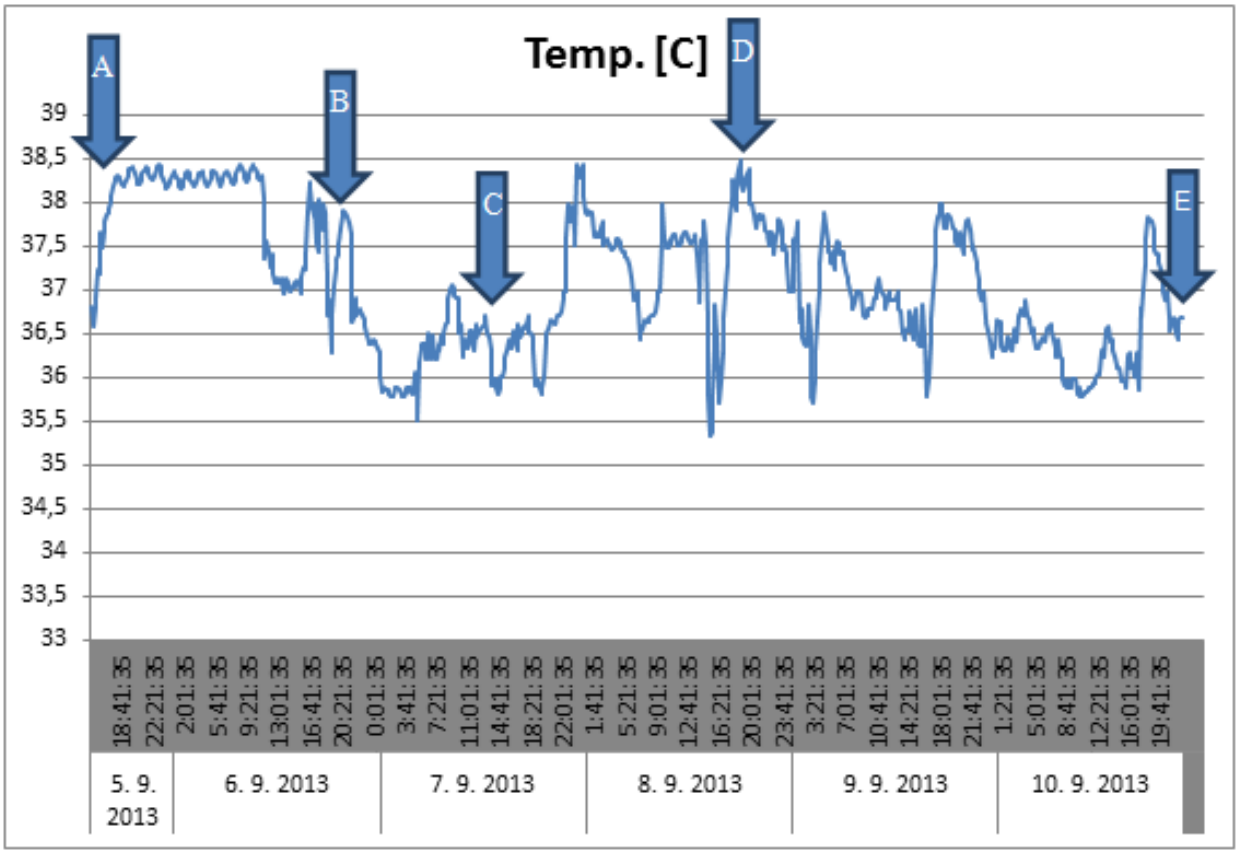

Figure 1. Documentation of the incubation conditions of the Enterococci and *E. coli* bacteria in incubator 1 in the hotel room in Dubai. Arrow A = begin of incubation time of ballast water uptake samples, test run 1; Arrow B = begin of incubation time of ballast water discharge samples, test run 1; Arrow C = begin of incubation time of ballast water uptake samples, test run 2; Arrow D = begin of incubation time of ballast water discharge samples, test run 2; Arrow E = end of incubation time.

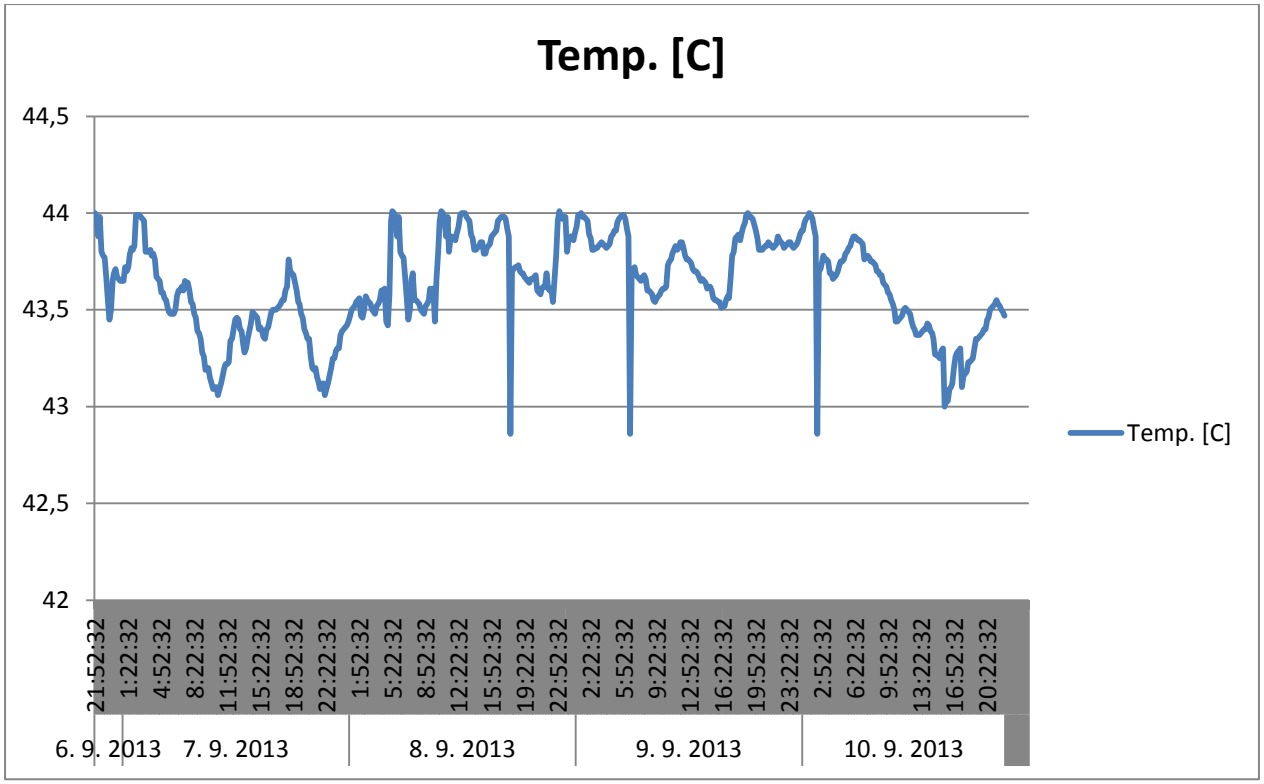

Figure 2. Documentation of the incubation conditions of the *E. coli* bacteria after transfer into Tryptophane broth in incubator 2 in the hotel room in Dubai.

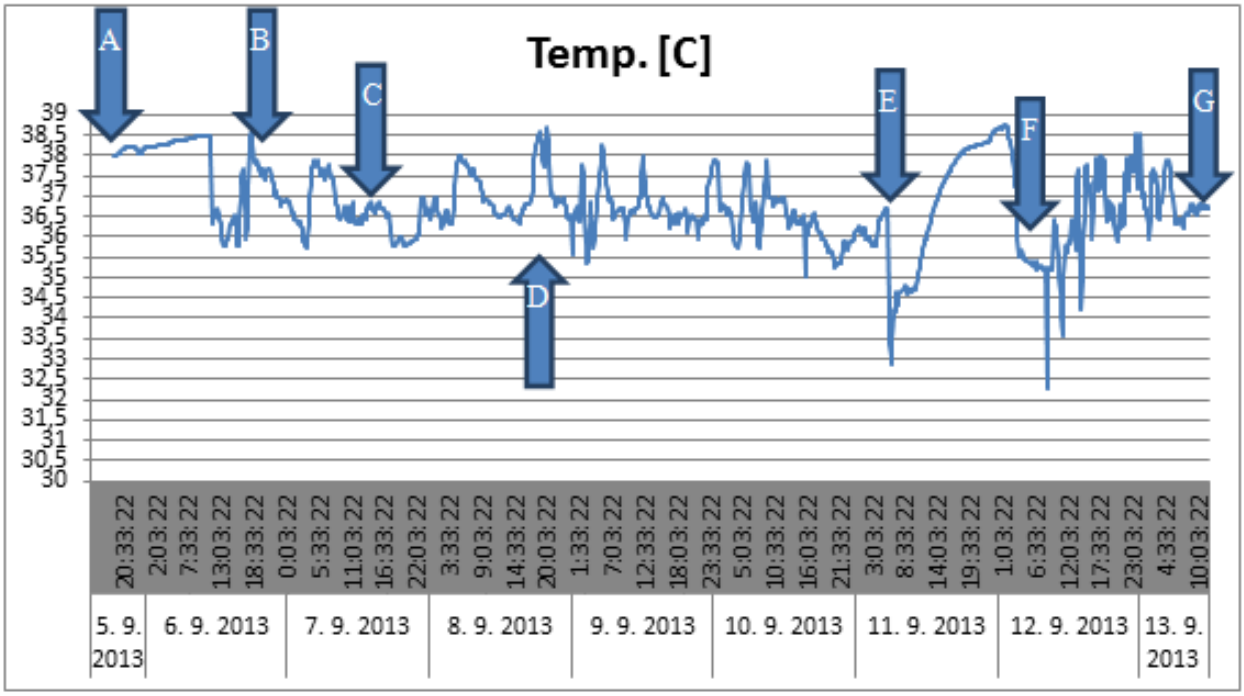

Figure 3. Documentation of the incubation conditions of the Cholera bacteria[1] in the hotel room in Dubai (incubator 3), and for the Cholera sample shipment including the waiting time at the Dubai airport, air travel and land-transport to IBEN, Bremerhaven, Germany. Arrow A = begin of incubation time of ballast water uptake samples, test run 1; Arrow B = begin of incubation time of ballast water discharge samples, test run 1; Arrow C = begin of incubation time of ballast water uptake samples, test run 2; Arrow D = begin of incubation time of ballast water discharge samples, test run 2; Arrow E = travel to Dubai airport, Arrow F = flight departure, and Arrow G = sample arrival at IBEN.

[1] Miller et al. (1984) wrote that toxigenic *V. cholerae* 01 survive for at least 70 days at 25 °C in solutions of sea salt. Lower temperatures were not tested. Pollitzer (1959) found that *V. cholerae* are unable to grow and utilize nutrients at temperatures below 10-12 °C. Further, it is known that Cholera bacteria grow best at temperatures above 17°C (http://www.medicalecology.org/water/cholera/cholera.htm, assessed 28.08.2012). According to Laboratory IBEN, Bremerhaven, Germany, the storage temperature should not drop much below 20 °C.

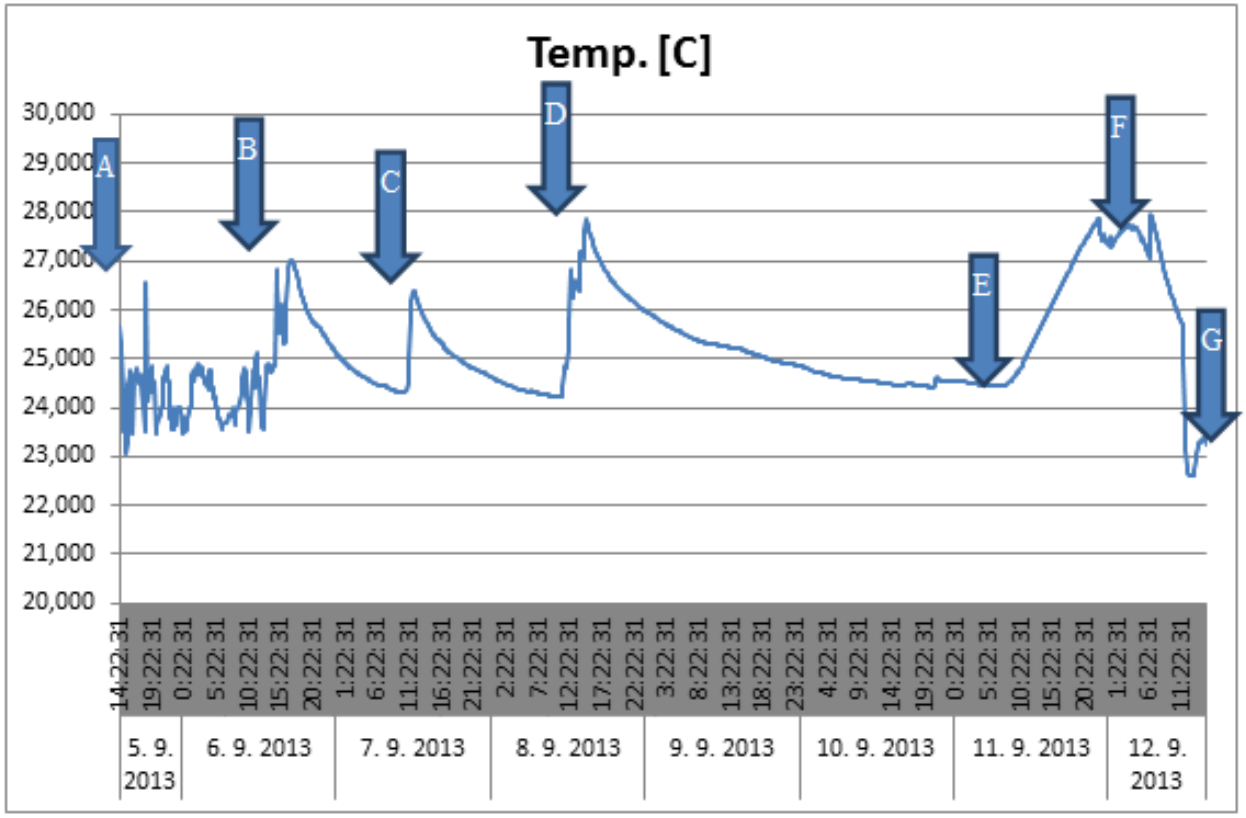

Figure 4. Documentation of the storage temperature of the phytoplankton samples, including the conditions in the hotel room in Dubai, waiting time at the Dubai airport, air travel and during land-transport to NIOZ, Texel, the Netherlands. Arrow A = begin of storage time for uptake water, test run 1; Arrow B = begin of storage time for discharge water, test run 1; Arrow C = begin of storage time for uptake water, test run 2; Arrow D = begin of storage time for discharge water, test run 2; Arrow E = travel to Dubai airport, Arrow F = flight departure, and Arrow G = sample arrival at NIOZ.

6. Discussion of the results

The Bawat ballast water treatment system has shown its performance ability to successfully treat organisms in ballast water as

- no living organisms above 50 micron in minimum dimension were found;
- no living organisms less than 50 micrometres in minimum dimension and greater than or equal to 10 micrometres in minimum dimension were found;
- no living toxicogenic *Vibrio cholerae* (serotypes O1 and O139) were found;
- the observed colony forming units of Enterococci and *E. coli* are well below the limits set in Regulation D-2 Ballast Water Performance Standard of the IMO Ballast Water Management Convention.

References

Gollasch, S. & Lüthke Christensen, O. 2013. Quality Management Plan and Quality Assurance Project Plan for Shipboard Tests of the BAWAT Ballast Water Management System. Version 5.2. 09.08.2013. 88 pp.

IMO 2004. International Convention on the Management of Ships' Ballast Water and Sediments. BWM/CONF/36, International Maritime Organization, London, United Kingdom, 36 pp.

IMO 2008. Guidelines for the Approval of Ballast Water Management Systems (G8). IMO Resolution MEPC.174(58), Marine Environment Protection Committee, International Maritime Organization, London, United Kingdom, 28 pp.

Miller, C., Drasar, B.S., Feachem, R., G. 1984. Response of toxigenic *Vibrio cholerae* 01 to physico-chemical stresses in aquatic environments. J. Hyg., Camb. 93, 475-495.

Pollitzer, R. 1959. Cholera. Monograph no. 43. Geneva: World Health Organization.

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25.06.2014

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Table of Content

1. Introduction

This is the summary report of the third test run to test the performance of the Bawat ballast water treatment system conducted in Port Khalid near Dubai (United Arab Emirates) in May 2014.

2. Biological sample processing

The samples were processed as described in the Bawat QAPP, Version 9.1, May 2014 (Gollasch & Lüthke Christensen 2014). For the organisms below 50 and above 10 µm in minimum dimension two sets of sub-samples were taken. The first set was handled as per Bawat QAPP (cooled to 4±2 °C), and the second was handled and transported at room temperature (not cooled).

3. Results

3.1. Environmental parameters

The sample processing revealed environmental parameters as expected to occur in the ballast water uptake region.

3.2. Biological results

The data presented below in the test cycle report are mean values of all measurements. For individual biological counts see the Appendices.

The number of organisms above 50 micron in minimum dimension in the uptake water met the challenge water conditions for tests of treatment systems as outlined in the IMO Test Guideline G8 (RESOLUTION MEPC.174(58) Adopted at IMO on 10 October 2008 GUIDELINES FOR APPROVAL OF BALLAST WATER MANAGEMENT SYSTEMS).

The PAM measurements conducted in the hotel room in Dubai and at NIOZ show very different results (see Appendix 7). This is especially the case for the samples with high cell content (uptake, discharge of the control test water).

Measured in the hotel in Dubai, according to the PAM measurements at all uptake samples and in the discharge control samples, algae were viable. In contrast the no algae viability was measured in the treated water on discharge.

At NIOZ the treated water on discharge cooled samples show very similar PAM values as measured in Dubai. However, the uptake before treated and control samples measured at NIOZ show PAM viability values in the range of 0.100, while the values at the uptake measured in Dubai were in the range of 0.500, which indicates many cells died before arriving at NIOZ. The discharge control samples measured at NIOZ showed approximately half the viability measurement as in Dubai (see Appendix 4).

As instructed by DNV, the uptake samples were stored in a fridge and the storage temperature was approximately as requested, i.e. oscillating around 4°C with +/- 2°C (see temperature logger results below). As previously recommended by Gollasch and David (Gollasch & David 2014), based on scientific literature data and own observations, a temperature drop from ambient conditions to a fridge (here a ca. 25 °C drop) is a temperature shock for algae used to live in the Dubai area at temperatures mainly above 24 °C, and with this causing death. This is likely what happened with the uptake before treated and control uptake and discharge samples.

It is interesting to note that the NIOZ counts revealed viable algae intake concentrations in the cooled samples just below the minimum required concentrations The samples kept at room temperature were measured to have more than 10 times higher viable algae concentrations.

This is different in the cooled treated water samples as here the PAM results are the lowest of all measurements so that the very few viable algal cells NIOZ counted in these samples are considered as in the process to die. In two samples (TR 1/2 and TR 2/2) NIOZ reported higher cell counts and this is considered as artefact/outliers, which may be justified by several reasons as reported by Louis Peperzak (NIOZ):

- The PAM measurements in Dubai and at NIOZ of these samples were among the lowest of all measurements, clearly indicating non-viable algae,
- Viable algae cells were reported in the heat-killed procedural control sample due to extracellular expression of green fluorescence, meaning that false positive counts in samples are possible, and
- Only 2 out of 9 samples are affected.

Further, no living zooplankton was found in the treated water samples.

The heat killed false positives are likely due to the fact that the stain bound to the cell wall of some algae, which was wrongly measured by the flow cytometer as viable cell. It should further be noted that, in case the outliers are ignored, the average number of viable phytoplankton cells would have been 3.5 ind/ml.

3.2.1. Test run 3

The test was done with a ballast water uptake on 08.05.2014, 09:15 – 12:18, in Khalid Port, near Dubai. Next day the ballast water was sampled at discharge: 09.05.2014, 11:37 - 14:41. The holding time of ballast water between uptake and discharge ca. 24 hours.

Water quality and number of organisms in uptake water (nd = not detected, P = phytoplankton, Z = zooplankton)

Parameter	**Unit**	**Uptake water**									
		before treatment			**aver.**	**IMO DNV**	**control**			**aver.**	**IMO DNV**
		#1	**#2**	**#3**	**#1-3**		**#1**	**#2**	**#3**	**#1-3**	
Temperature	°C	31.3	31.0	31.0	-	-	31.1	31.1	31.1	-	-
Salinity	PSU	40.5	40.6	40.6	-	-	40.5	40.5	40.4	-	-
POC **	mg/l				-	-				-	-
TSS **	mg/l				-	-				-	-
Sample vol. >50 µm	Litres	1120	1166	1310	1199	>1000	1290	1215	1201	1235	>1000
Sample vol. 50-10 µm	Litres	5-6	5-6	5-6	5-6	>5	5-6	5-6	5-6	5-6	>5
Sample vol. bacteria	Litres	1	1	1	1	>0,5	1	1	1	1	>0,5
Organisms >50µm	org./1m³	4107	3997	2450	3518	>90	2357	2560	2598	2505	>90
	time [h]	<6h	<6h	<6h		<6h	<6h	<6h	<6.3h	<6.1h	<6h
Organisms 10-50µm*	P org./1ml cool	76.0	45.3	89.3	70.2	>90	100.3	63.0	96.3	86.6	>90
	P org./1ml room temp.	257.1	695.2	1631.7	861.4		1414.3	1563.5	2160.3	1712.7	
	Z org./1ml	1.5	2.6	2.1	2.1		1.3	1.6	2.0	1.6	
	time [h]	PAM <6h, NIOZ 11.05.14				<6h	PAM <6h, NIOZ 11.05.14				<6h
Escherichia coli	cfu/100ml	15	70	190	91.7	-	150	70	170	130.0	-
	time [h]	<4h	<4h	<4h	<4h	<4h	<6h	<6h	<6h	<6h	<6h
Intestinal *Enterococci*	cfu/100ml	80	80	70	76.7	-	140	120	160	140.0	-
	time [h]	<4h	<4h	<4h	<4h	<4h	<6h	<6h	<6h	<6h	<6h
*Vibrio cholerae****	cfu/100ml	nd	nd	nd	nd	-	nd	nd	nd	nd	-
	time [h]	IBEN 14-27.05.14					IBEN 14-27.05.14				

* Analyzed at NIOZ, Texel, The Netherlands, except zooplankton which was processed in the hotel room in Dubai. Please note that one set of samples was kept cool and an additional set was stored at room temperature. ** Analysed at IBEN, Bremerhaven, Germany. All PAM samples were prepared within 6 hours after sampling ended (dark adaptation), uptake samples were measured <6h.

Water quality and number of organisms in discharge water (nd = not detected, P = phytoplankton, Z = zooplankton)

Parameter	**Unit**	**Discharge water**															
		control			**aver.**	**IMO DNV**	**treated**									**aver.**	**IMO DNV**
		#1	**#2**	**#3**	**#1-3**		**#1**	**#2**	**#3**	**#4**	**#5**	**#6**	**#7**	**#8**	**#9**	**#1-9**	
Temperature	°C	29.8	29.7	29.8	-	-	41.0	41.0	41.0	41.0	41.0	41.0	41.1	41.1	41.1	-	-
Salinity	PSU	40.3	40.4	40.5	-	-	40.4	40.4	40.4	40.3	40.3	40.3	40.4	40.4	40.4	-	-
POC **	mg/l				-	-										-	-
TSS **	mg/l				-	-										-	-
Sample vol. >50 µm	Litres	1204	1205	1200	1203	>1000	1735	1752	-	1803	1720	-	1586	1681	-	1713	>1500
Sample vol. 50-10 µm	Litres	5-6	5-6	5-6	5-6	>5	5-6	5-6	5-6	5-6	5-6	5-6	5-6	5-6	5-6	5-6	>5
Sample vol. bacteria	Litres	1	1	1	1	>0.5	1	1	1	1	1	1	1	1	1	1	>0,5
Organisms >50µm	org./1m³	1744	2539	2467	2250	>10	nd	nd	-	nd	nd	-	1.7	nd	-	0.2	<10
	time [h]	<6h	<6h	<6.3h	<6.1h	<6h	<6h	<6h	-	<6h	<6h	-	<6h	<6h	-	<6h	<6h
Organisms 10-50µm*	P org./1ml cool	19.0	39.7	31.7	30.1	>10	2.7	28.7	6.0	2.0	28.0	2.0	2.0	4.0	6.7	3.5***	<10
	P org./1ml room temp.	325.4	849.2	525.4	566.7		0.0	3.2	0.0	11.1	20.6	1.6	6.3	9.5	1.6	6.0	
	Z org./1ml	0.8	0.7	0.9	0.8		nd	nd	nd	nd	nd	nd	nd	nd	nd	<6h	
	time [h]	PAM <8h, NIOZ 11.05.14				<6h	PAM <8h, NIOZ 11.05.14										<6h
Escherichia coli	cfu/100ml	nd	nd	nd	nd	<250	20	10	20	20	10	nd	nd	nd	nd	8.9	<250
	time [h]	<5h	<5h	<5h	<5h	<5h	<6h	<6h	<6h	<6h	<6h	<6h	<6h	<6h	<6h	<6h	<6h
Intestinal *Enterococci*	cfu/100ml	nd	nd	10	3.3	<100	10	10	10	20	50	20	nd	20	10	16.7	<100
	time [h]	<5h	<5h	<5h	<5h	<5h	<6h	<6h	<6h	<6h	<6h	<6h	<6h	<6h	<6h	<6h	<6h
*Vibrio cholerae****	cfu/100ml	nd	nd	nd	nd	<1	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	<1
	time [h]	IBEN 14-27.05.14					IBEN 14-27.05.14										

* Analyzed at NIOZ, Texel, The Netherlands, except zooplankton which was processed in the hotel room in Dubai. Please note that one set of samples was kept cool and an additional set was stored at room temperature. ** Analysed at IBEN, Bremerhaven, Germany. All PAM samples were prepared within 6 hours after sampling ended (dark adaptation), discharge samples were measured <8 h. *** As recommended by NIOZ, the two outliers in sample # 2 and 5 were excluded from the mean value. When included the mean value is 9.1 ind./ml.

4. Remarks

For test run 3:

- All technical requirements, such as minimum water volumes of the water to be sampled etc. for a valid test according to IMO Guideline G8 or as agreed with DNV prior the tests were met.
- For the organisms above 50 µm the mean values of the minimum organism intake concentrations and, at discharge of the treated water, D-2 was met.
- For the organisms below 50 and above 10 µm in minimum dimension two sets of samples were taken, prior analysis one set was cooled and a second set was stored at room temperature. Both sets were processed at NIOZ in parallel. All samples were processed by flow cytometry and the cooled samples were processed by using a FACS Canto machine and the samples kept at room temperature by an Accuri C6 machine.
- In the cooled samples the mean values of the minimum organism intake concentrations for organisms below 50 and above 10 µm in minimum dimension where below the required values set by IMO, but at discharge of the treated water, D-2 was met when considering the mean value.
- For the samples of organisms below 50 and above 10 µm in minimum dimension stored at room temperature both uptake samples and also the discharge control water contained much higher viable organisms compared to the cooled samples, and at discharge of the treated water, D-2 was met when considering the mean value.
- For the indicator microbes *Escherichia coli*, Enterococci and *Vibrio cholerae* D-2 was met at discharge.
- All samples, with the exception of two (see below), were prepared for processing in the hotel room in Dubai according to the QAPP and G8 paragraph 2.3.34: *The samples should be analysed as soon as possible after sampling, and analysed live within 6 hours or treated in such a way so as to ensure that proper analysis can be performed*. For two samples this time limit could not be met. Here the time between the end of the sampling event and the end of the sample processing was ca. 6 hours and 20 minutes. This refers to the last replicates of both control samples (uptake and discharge).
- For the Dubai PAM measurements the phytoplankton samples were completed in maximum 8 hours after sampling.
- Although the water temperature during the uptake sampling events was high (ca. 30 C), the phytoplankton species were stored in the fridge as required by DNV. The shipment of the phytoplankton samples was arranged by a courier service and the samples were shipped in a cooling box with pre-frozen ice packs (see temperature logger data in the next chapter). The samples arrived on 10.05.2014 in the evening and were processed at NIOZ the next day. The second set of samples was held at room temperature and shipped at the same time, packed in same way in separate transport boxes without any cooling elements, so that all samples arrived at NIOZ in one shipment.
- The phytoplankton samples of the uptake were processed at NIOZ approximately 3 days after the sampling event had ended and the discharge samples after 2 days using a flow cytometer with FDA/CMFDA as agreed with DNV.

- The blank *E. coli* and Enterococci media experiment during both the uptake and discharge test was negative, which indicates that the distilled water and the selective bacteria media were not contaminated, as well as the bacteria processing in the hotel room did not cause any bacteria contamination.

5. Temperature logger measurements

To document the sample storage, transport and bacteria incubation conditions temperature loggers were used. The loggers were set to take a temperature measurement every 10 minutes and the following figures show the results. Arrows are used to indicate approximate times for the events as stated in the figure capture. It should be noted that some temperature drops occurred due to opening the incubators for bacteria inspections. This was only done at the end of the incubation time so that the short temperature drop has no effect on the incubation.

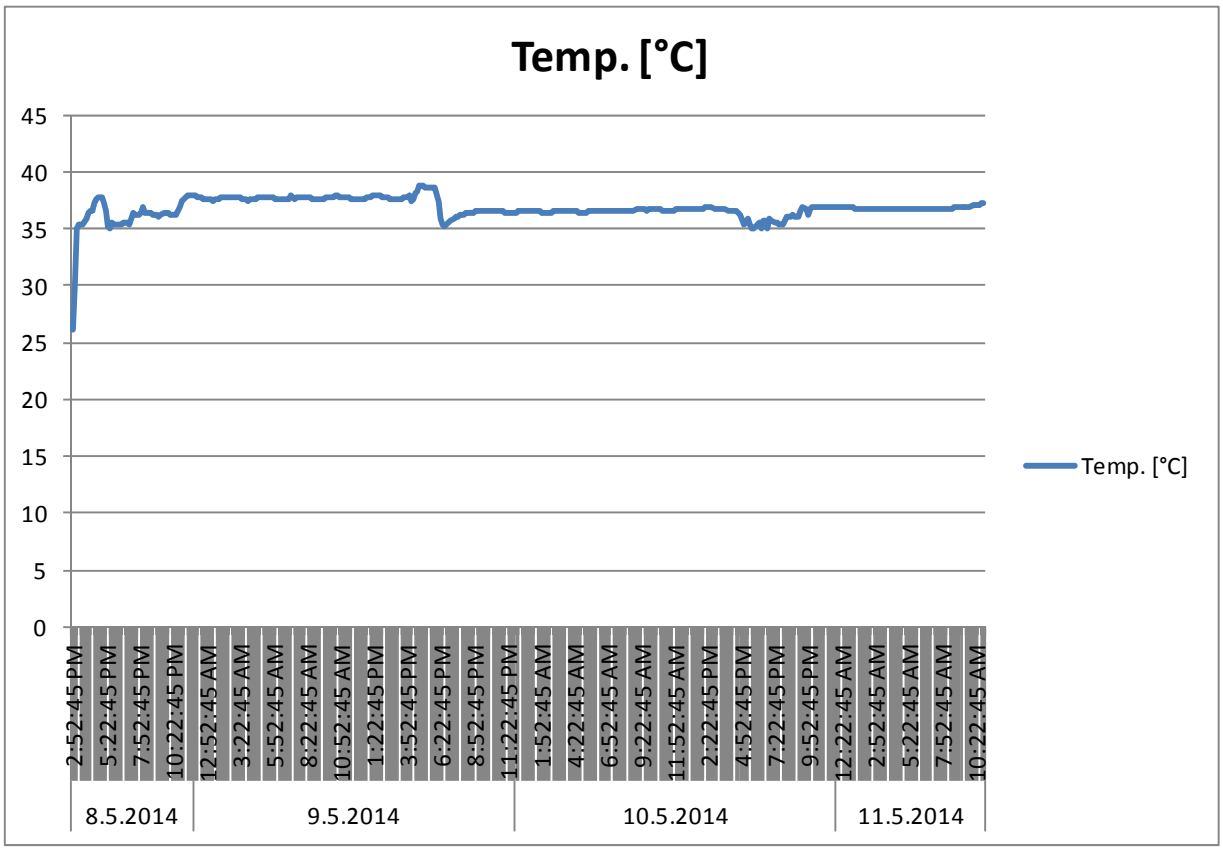

Figure 1. Documentation of the incubation conditions of the Enterococci and *E. coli* bacteria in incubator 1 (logger nr. 4) in the hotel room in Dubai.

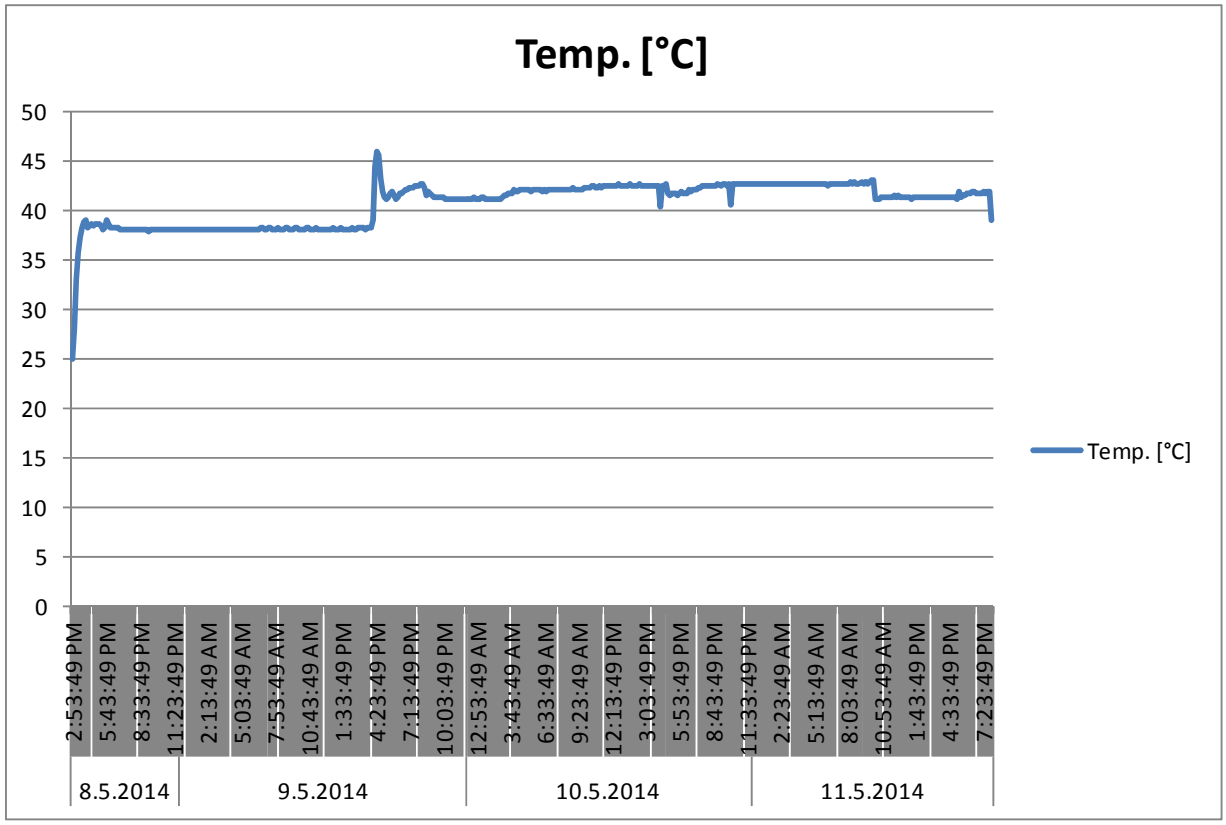

Figure 2. Documentation of the incubation conditions of the *E. coli* bacteria after transfer into Tryptophane broth in incubator 4 (logger nr. 6) in the hotel room in Dubai.

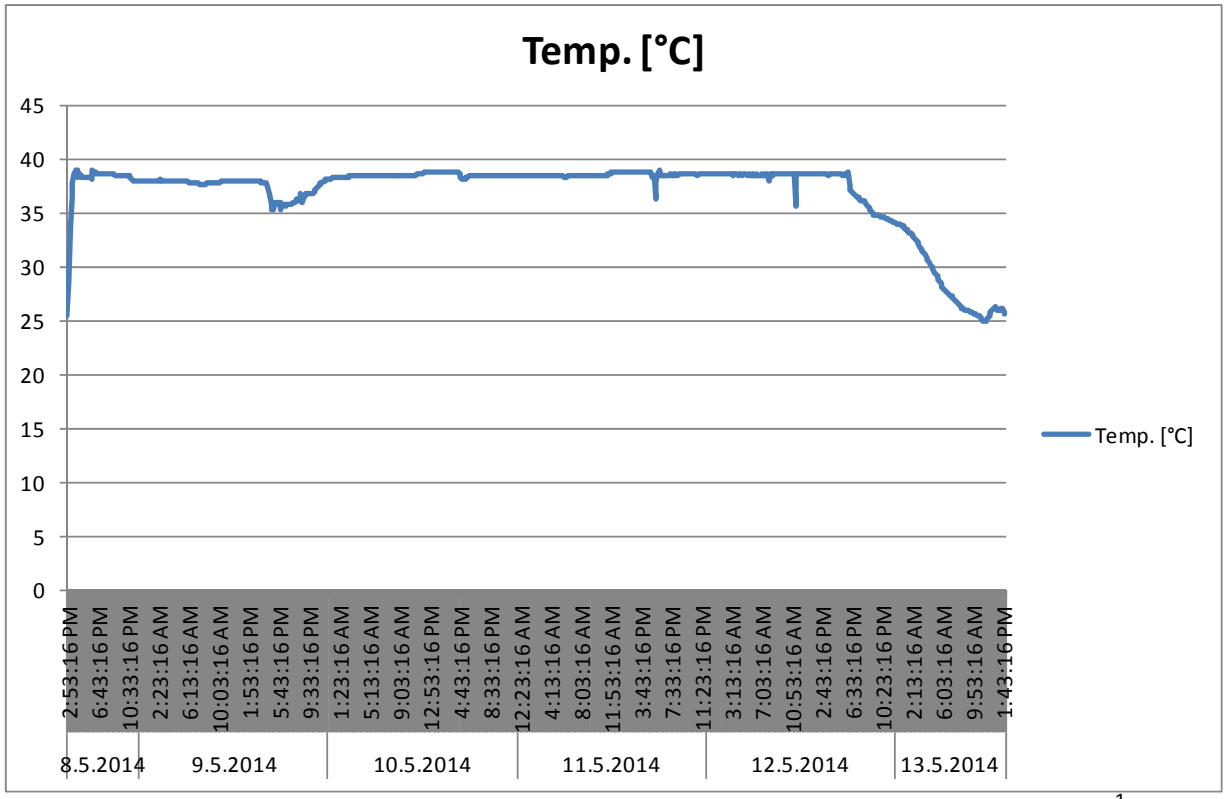

Figure 3. Documentation of the incubation conditions of the Cholera bacteria[1] in the hotel room in Dubai (incubator 2, 3) (logger nr. 5), and for the Cholera sample shipment including the waiting time at the Dubai airport, air travel and land-transport to IBEN, Bremerhaven, Germany.

[1] Miller et al. (1984) wrote that toxigenic *V. cholerae* 01 survive for at least 70 days at 25 °C in solutions of sea salt. Lower temperatures were not tested. Pollitzer (1959) found that *V. cholerae* are unable to grow and utilize nutrients at temperatures below 10-12 °C. Further, it is known that Cholera bacteria grow best at temperatures above 17°C (www.medicalecology.org/water/cholera/cholera.htm, assessed 28.08.2012). According to Laboratory IBEN, Bremerhaven, Germany, the storage temperature should not drop much below 20 °C.

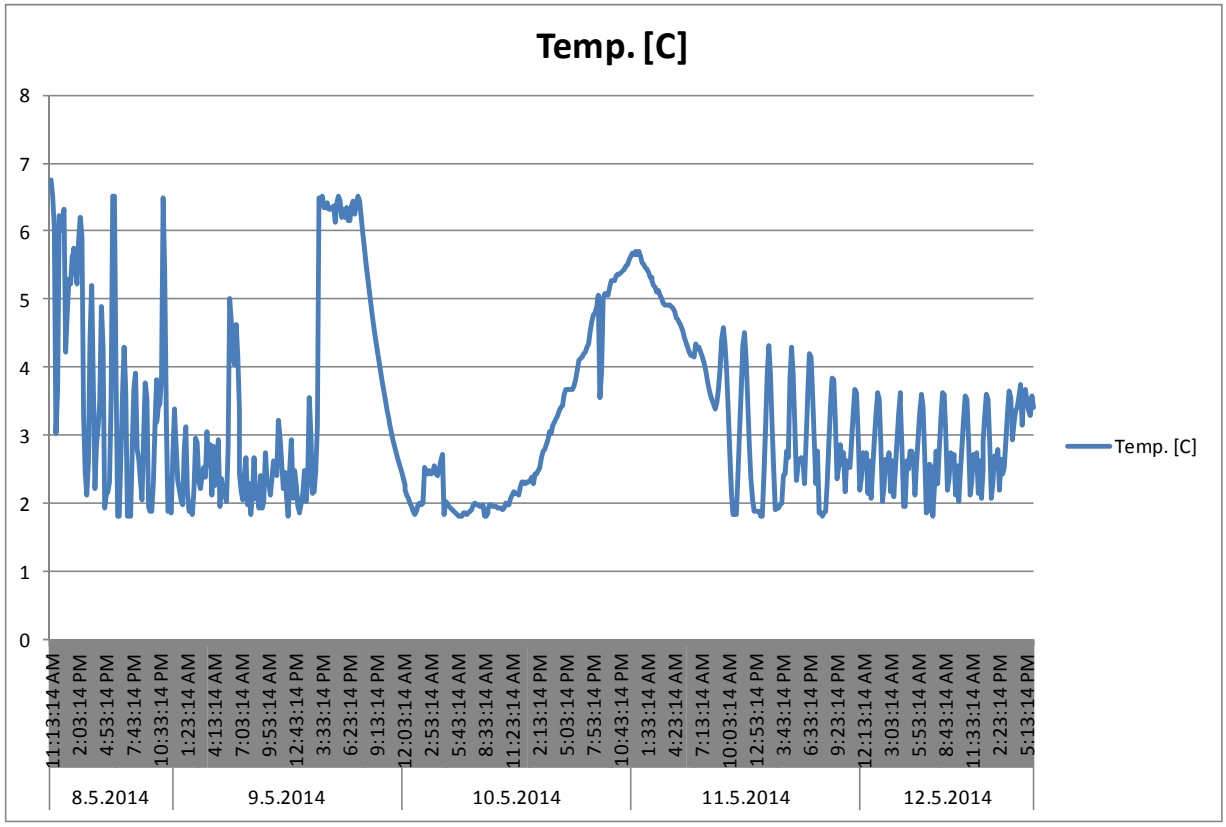

Figure 4. Documentation of the storage temperature of the cooled phytoplankton samples (logger nr. 2), including the conditions in the fridge in Dubai, air travel and during land-transport to NIOZ, Texel, the Netherlands.

6. Discussion of the results

Though some required parameters were no fully met, the Bawat ballast water treatment system has shown its performance ability to successfully treat organisms in ballast water as in the treated ballast water on discharge:

- only one living organisms above 50 micron in minimum dimension was found. When considering the volume of the concentrated sample and sub-sample volume analysed, this results in a mean value of 0.19 ind./m^3;
- only few organisms less than 50 micrometres in minimum dimension and greater than or equal to 10 micrometres in minimum dimension were found, and some of these findings are considered by NIOZ as an artefact/outlier;
- the uptake samples and control discharge samples for organisms less than 50 micrometres in minimum dimension and greater than or equal to 10 micrometres in minimum dimension stored in cooled conditions showed multiple times lower viable cell counts compared to the samples for this organism group when stored at room temperature;
- no living toxicogenic *Vibrio cholerae* (serotypes O1 and O139) were found;
- the observed colony forming units of Enterococci and *E. coli* are well below the limits set in Regulation D-2 Ballast Water Performance Standard of the IMO Ballast Water Management Convention.

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